Comparison of Protocols for Isolating Large Insert Clone DNA that is Suitable for High Throughput Library Construction

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JGI has been generating sequences from numerous large insert size clones each year. These targeted sequences allow collaborators to study regions of interest without having to sequence the entire genomes. As part of the assembly QC process, JGI would also sequence a set of fosmids or BACs randomly selected from all eukaryotic genomes to aid in the assembly. Last year JGI has isolated DNA from about 1,200 large insert clones, constructed libraries from each clone, and sequenced them.

We have been using Qiagen® Plasmid Maxiprep protocol to isolate DNA from BAC/fosmid clones, which is laborious and time consuming. In the attempt to find a more efficient way of isolating DNA from these clones, we have compared several protocols including the GenElute™ HP Plasmid Maxiprep from Sigma, the BACMAX™ DNA Purification Kit from Epicentre, and the Edge BioSystems FosPrep™ 96 Fosmid Prep. An overview of each protocol will be presented. We will also provide the comparison of costs, amount of time, DNA yield, quality, and the suitability for library construction from using these DNA isolation protocols.

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